Chapter

Modified (2′,5′)Oligonucleotides: The Influence of Structural and Steriochemical Factors on Biological and Immunotropic Activity

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Abstract

The synthesis of a large number of analogs of natural 2-5A and the results of studies to clarify the relationship between the structure and spatial organization (stereochemistry) and the biological properties of analogs 2-5A have convincingly demonstrated that by changing the structure and/or stereochemistry of molecules, it is possible to achieve either strengthening of known properties or giving new ones. The replacement of the adenosine fragment with 1-deazaadenosine (c^1A) or 3-deazaadenosine (c^3A) at various positions of the 2-5A chain demonstrated the role of each of the nitrogen atoms of the adenine heterocycle in the processes of binding and activation of RNase L. The use of conformationally rigid fluorodeoxyadenylates in enzymatic reactions made it possible to differentiate the role of structural and stereochemical factors and demonstrate the influence of molecules' stereochemistry on their biological properties. Oligomers with *rib*o-[(2′,5′)A_2A_{RA}] and *lixo*-[(2′,5′)A_2A^{LA}] conformation in the (A3) terminal fragment showed activity against diseases associated with disorders of T-cell immunity, autoimmune diseases, viral infections, lymphocytic malignant transformations, prevention of transplant rejection after bone marrow transplantation and, possibly, in the treatment of complications associated with the reaction of the transplanted tissue and the recipient's tissue.

Keywords: 2-5A, (2′,5′)oligoadenylates analogs, RNase L, stereochemistry, NK-cells, lytic activity, phagocytosis, immunosuppressive activity

1. Introduction

(2′,5′)Oligoadenylates represent one of the elements of cellular endogenous antiviral defense which is induced by interferon in response to RNA molecules synthesized in virally infected cells [1, 2]. The key role of 5′-triphosphorylated (2′-5′) oligoadenylates, 2-5A [ppp(A2′p5′)$_n$A, n = 2÷15; mainly, trimer, n = 2] (**Figure 1**), in the antiviral effect of interferon is widely recognized. Oligoadenylates bind and then activate 2-5A-dependent endoribonuclease (RNase L) contributing to the hydrolysis

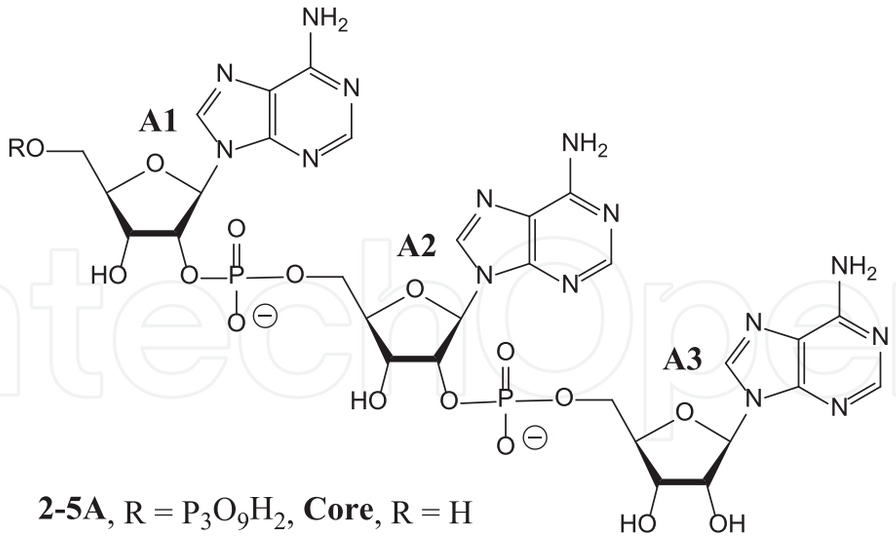


Figure 1.
*Structures of 5′-phosphates of the trimer 2′,5′-oligoadenylic acid [pppA2′p5′A2′p5′A (***2-5A***)] and dephosphorylated analog [***Core***].*

of viral mRNA and, consequently, the inhibition of viral protein synthesis [3–5]. Low molecular weight oligomers are present in nanomolar (10^{-9} M) concentration in living organisms and play a crucial role in the antiviral effects of interferon, maintaining early pregnancy, endochondral ossification, myogenesis, neuronal differentiation, apoptosis, heat-shock response, etc. Findings obtained from biochemical examinations of the cellular endogenous antiviral defense aroused great interest in analyzing of the 2-5A system general functioning mechanisms, as well as biochemical role played by its specific components [6–10].

However, 2-5A is rapidly cleaved by 2′-5′-phosphodiesterase [11, 12]. Moreover, the molecule has a large negative charge and is incapable to penetrate the cell membrane. These shortcomings can be eliminated by modifying the molecule while retaining the ability to bind to RNase L. Modifications can be made to the base, sugar, or phosphate fragments. But the development of new modified oligonucleotides is a challenge due to chemical, electronic, and steric problems. Additionally, the scope of application of oligonucleotides can be expanded through their structure chemical modification. Therefore, synthetic oligonucleotides are currently used for a wide range of purposes including biotechnology, molecular biology, diagnostics, and therapy.

In this review, the main focus will be on ppp(A2′p5′)$_2$A and dephosphorylated trimers modified in the nucleotide part, and the analysis of effects of structural and stereochemical characteristics on some biological properties including immunotropic activity.

2. (2′-5′)Oligoadenylates modified on the heterocyclic base

The contribution of various functional groups of each individual 2-5A nucleoside moiety is highly specific to both RNase L binding and activation. There have been scientific publications in which ppp(A2′p5′)$_2$A or trimer thereof were modified in the nucleotide part and subsequently assessed for their sustained ability

to activate endonuclease. As a result of these studies, structural requirements emerged that are important for RNase L activation (reviewed in [4, 13]). Adenine bases were modified in several positions [14, 15], or even substituted by other base moieties [15, 16]. Substitution of H-8 in adenine with a Br atom [17], a methyl group [18], or a hydroxyl group [19] seems to change mainly the base-to-ribose orientation, which may have implications for endonuclease binding capacity. Despite the assumption of minor role of the central adenosine residue [14], its substitution by uridine reduces binding and activation significantly [16]. These studies have generally shown that the presence of adenosine in all positions of the 2-5A chain is crucial for the mediator activity and minimal modifications of heterocyclic bases might be acceptable.

Substitution of the adenosine moiety with 1-deazaadenosine (c^1A) or 3-deazaadenosine (c^3A) at different positions of the 2-5A chain first allowed to establish the role played by each of the adenine heterocycle nitrogen atoms in RNase L binding and activation [20–22].

The 2-5A analogs where adenosine was sequentially substituted by inosine show that (A1) NH_2–C6–N1 moiety of the adenosine residue is critical for binding to RNase L. The same 2′-terminal (A3) adenosine residue moiety is required for the enzyme activation [14]. However, during the examination of the structure/biological activity dependence, a substitution of adenosine by inosine shall be considered as it causes two simultaneous alterations of the chemical structure including the transformation of adenine primary exocyclic 6-amino group into a keto function and the transformation of adenine N1 nitrogen atom into a NH part of hypoxanthine (**Figure 2**). Thus, previous data did not allow us to draw a definite conclusion as to what caused the loss of binding and/or activating ability of the above analogs substituted for inosine—the loss of exocyclic adenosine NH_2-C6 group or transition of the tertiary adenine N1 nitrogen atom to the secondary atom in hypoxanthine? The use of 2-5A 1-deazaadenosine analogs resolved the above ambiguity since the substitution of adenosine by 1-deazaadenosine removes solely the adenine N1 nitrogen atom turning it into a CH moiety yet leaving the exocyclic primary amino function unchanged (**Figure 2**) [20, 21].

The pA(c^1A)A deaza analog containing c^1A in the trimer middle chain link was highly active which is consistent with data on the inosine analog pA(**I**)A activity [14]. Substitution of (A1) moiety with both c^1A, p(c^1A)A_2, and inosine, p(I)A_2 reduces the ability to activate RNase L by about a factor of 33. So the loss of activity is due to the absence of tertiary N1 nitrogen atom and not the absence of the exocyclic amino group. These results are consistent with data provided in [23] which showed that 2-5A

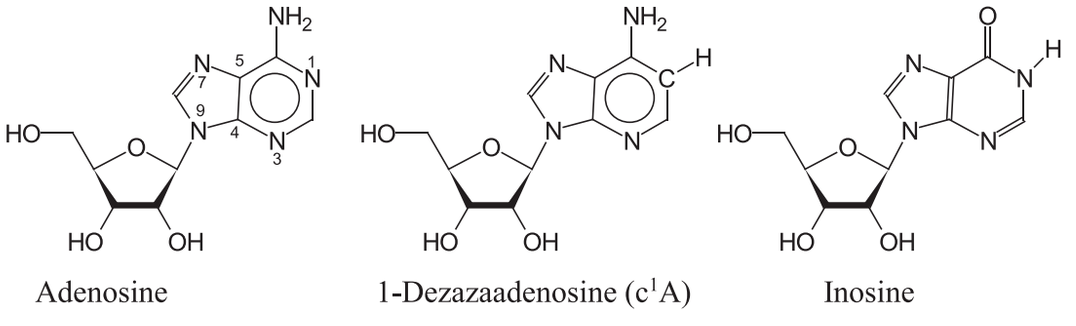


Figure 2.
Structural formulas of adenosine (A), 1-dezazaadenosine (c^1A), and inosine (I).

analog containing 1-(β-D-ribofuranosyl)-1H-1,2,4-triazolo-3-carboxamide (ribavirin) in (A1) moiety activates recombinant RNase L from human CEM cell extracts more effectively compared to the parent trimer. The carboxamide group of ribavirin is probably better in mimicking NH_2–C6–N1 moiety 5′-terminal adenosine compared to c^1A. It should also be noted that N1 of (A1) nitrogen atom of adenosine moiety can be pivotal in binding (2′-5′) oligomers with RNase L. Deazaadenosine analog $pA_2(^1A)$ showed a 20-fold increase in activating ability compared to pA_2(**I**) and as much as a 5-fold decrease compared to the parent tetramer pA_4. Obviously, the NH_2-C6 exocyclic amino group of 2-5A (A3) adenosine moiety is responsible for the conformational "switch" that induces activation of RNase L.

It was shown that substitution of 5′-terminal or 2′-terminal adenosine for c^3A produced respective analogs including p5′(c^3A)2′p5′A2′p5′A and p5′A2′p5′A2′p5′(c^3A) which were not inferior to the parent tetramer in activating RNase L ($EC_{50} \leq 1$ nM) [22]. In contrast, p5′A2′p5′(c^3A)2′p5′A showed a reduced ability to activate RNase L ($EC_{50} \leq 10$ nM). These data are consistent with substantial stereochemical discrepancies between A2′p5′(c^3A)2′p5′A and the parent core (2′,5′) trimer whereas a specific recognition of N3 atom of mid-adenosine (A2) is unlikely. The extensive conformational analysis of c^3A-substituted core trimers compared to the initial parent core trimer showed close stereochemical resemblance between the parent core trimer and (c^3A)2′p5′A2′p5′A and A2′p5′A2′p5′(c^3A) analogs which is a strong evidence of syn orientation of the base with respect to the glycoside bond. Conversely, A2′p5′(c^3A)2′p5′A analog deviated rather significantly from the spatial arrangement of the parent core trimmer. The extensive conformational analysis of the c^3A-substituted core trimers versus the parent natural core trimer displayed close stereochemical similarity between the natural core trimer and (c^3A)2′p5′A2′p5′A and A2′p5′A2′p5′(c^3A) analogs, thereby strong evidences for the syn base orientation about the glycosyl bond of the c^3A residue of the latter were found. On the contrary, an analog A2′p5′(c^3A)2′p5′A displayed rather essential deviations from the spatial arrangement of the parent natural core trimer.

Synthesis data of 2-5A analogs containing 6-(benzylamino)purine riboside (AdoBn), a nucleoside with cytokinin activity are of interest. The second type of modification of the heterocyclic base was a replacement for virazole (ribavirin), a synthetic nucleoside that exhibits a wide range of antiviral activity against a wide variety of viruses. Compounds with high antiviral activity were found among the obtained oligomers. Studies of biological properties exhibited by these 2-5A analogs [23, 24] showed that these compounds had HIV-1 reverse transcriptase inhibitory and recombinant human ribonuclease L activity [25]. Specifically, trimers containing AdoBn at any positions of the oligonucleotide chain have been shown to impede the HIV-1-induced formation of syncytium by 1500 times (vs. three times for 2′,5′A_3 parent trimer). It is also in evidence that all virazole-containing trimers at a concentration of 300 μmol have been shown to inhibit HIV-1 reverse transcriptase by 99.5–99.7% (33% for parent trimer). The ability of AdoBn-containing compounds to inhibit this enzyme is based on the position of the modified nucleoside unit in the oligomer chain and is greatest for the trimer containing the above moiety in the 5′-terminal position. The ability of the studied 2′,5′-oligonucleotides to activate recombinant human ribonuclease L defined as the percentage of poly(U)-3′-[^{32}P]pCp hydrolysis in the presence of these compounds also depends on the structure of these oligoadenylates. Thus, the trimer containing a virazole moiety in the 5′-terminal position of the chain inhibited ribonuclease L by 87.7%

(50% for the parent trimer). AdoBn-containing compounds as the 5′-terminal and central link—by 37.4% and 34.8%, respectively, whereas the heterobase modified 2′-terminal unit of oligoadenylates resulted in a complete loss of ability to activate ribonuclease L [25].

3. (2′-5′)Oligoadenylates modified in the carbohydrate moiety

3.1 The role of structural and stereochemical factors in binding and activation of RNase L

The synthesis of parent 2-5A analogs modified in the carbohydrate moiety and the study of their physicochemical and biological properties helped to establish the role of structural and stereochemical factors of this unique class of cell biomolecules.

The substitution of ribose 3′-OH groups of ppp(A2′p5′)$_2$A with hydrogen proved that 3′-OH groups of (A2) moiety are essential for biological activity (**Figure 3**). However, this group is perhaps required for degradation by 2′-5′-phosphodiesterase since (A2′p5)$_2$A with (A2) substituted by xyloadenosine was resistant to 2′-5′-phosphodiesterase activity [26]. For 2′-5′-phosphodiesterase, 3′-OH groups of the second residue and free 2′- and 3′-OH groups of the 2′-terminal residue [27–30] are required.

The results of examinations to assess relationship between the structure and spatial arrangement (stereochemistry), on the one hand, and the biological properties of 2-5A analogs, on the other hand, clearly showed that the known properties can be enhanced or new properties can be added by changing the structure and/or stereochemistry of their molecules. For example, the use of fluorodeoxyadenylates, parent oligomer analogs, in which adenosine moieties are consecutively substituted by a conformationally rigid fluoronucleoside molecule for enzyme reaction tests allowed to differentiate the role of structural and stereochemical factors and demonstrate the effect of molecule stereochemistry on their biological properties [31].

Substitution of the 3′-hydroxyl group of adenosine furanose ring with a fluorine atom in two different configurations, *ribo* (A_F) and *xylo* (A^F), contributes to the overriding population of different conformations of the pentofuranose moiety due to stereochemical differences in the gauche effects of the fluorine atom and other electronegative substituents of furanose rings. It was demonstrated that the conformational features of individual fluoronucleosides A_F and A^F, which are mainly in the

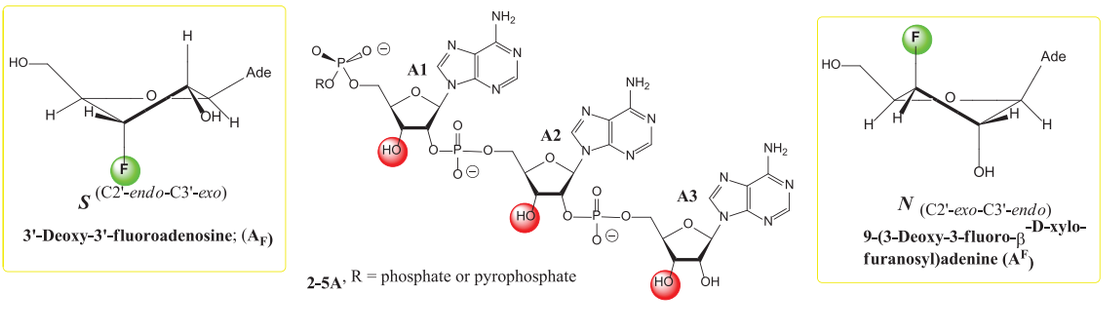


Figure 3.
Stereochemical structures of 3′-fluoro-ribo-adenosine (A_F), 3′-fluoro-xylo-adenosine (A^F), and the structure of 5′-phosphates of the 2′,5′-oligoadenylic acid trimer.

N- or S-conformation, respectively, are also preserved in the respective fluoronucleoside moiety of the trimer. These datademonstrated that the fluorine atom present in the carbohydrate part of the molecule is a key factor of the conformation of these molecules [31].

Stereochemical features of 2-5A *xylo*- and *ribo*-fluorodeoxy analogs make these compounds unique stereochemical analogs of the parent oligomer, which enables to discriminate the role of structural and stereochemical factors in biological processes. Indeed, on the one hand, a pair of A_F and A^F substituted analogs in a certain position of the chain is structurally related to 3′-deoxyadenosine (cordycepin) analogs, while on the other hand, the sugar rings A_F and A^F are in different conformations, S and N, respectively. These stereochemical differences between A_F and A^F are preserved when included in the (2′-5′) oligomer molecule instead of adenosine, and determine largely its stereochemistry. The *syn*↔*anti* orientation change of the heterocyclic base of A_F and A^F fluoronucleosides with prevalent syn- or anti-conformers, respectively, results in a variety of stacking interactions of heterocyclic bases and thereby changed oligonucleotide chain conformation as a whole. The introduction *of xylo*-fluoronucleoside (A^F) into the central (A2) 2-5A moiety produced an oligomer more resistant to (2′,5′)-phosphodiesterase compared to the parent trimer. At the same time, the presence of 3′-deoxy-, 3′-fluoro-*xylo*(A^F), or 3′-fluoro-*ribo*(A_F) adenosine in the (A3) moiety of oligoadenylate produced different degrees of hydrolysis despite their structural resemblance suggesting the role of the spatial arrangement of the molecule in the recognition thereof by phosphodiesterase active site [32].

The study of the ability of 2-5A fluorodeoxyanalogs to bind and activate RNase L showed that the analog whose fluorine atom in the *xylo*-configuration in the mid-moiety is nine times more active than the parent mediator, 2-5A and about 280 times more effective than isomeric *ribo*-trimer [32–34]. The results suggest that *anti*↔*syn* stereochemical differences between pppAA(A_F) and pppAA(A^F), on the one hand, and related 8-bromo- and -methyl-analogs, on the other hand, cause differences in the degree of RNase L activation. Data presented in [32–34] suggest the *syn*-orientation is a major contribution to this process. The pppA(A^F)A_F poor ability to activate RNase L supports this assumption.

It is noteworthy that 2-5A fluorine-substituted analogs are important for the analysis of stereochemical patterns of RNase L activation. The *syn*-orientation of the base both at 5′- terminal of the oligomer and in the central site and the prevalent *N*-conformation of pentofuranose residues are probably required to form a productive complex between the enzyme, mediator, and substrate. In addition, the *syn*-orientation of the base in (A3) moiety of the oligomer is positive for RNase L activation. These reasonably substantiated assumptions are quite paradoxical and a question suggests itself: could this unusual *syn*-orientation of bases being in the transient state during RNase L activation be the second - after the unusual (2′-5′) phosphodiester bond in the oligomer—unique property of this mediator?

The *syn*-orientation of the heterocyclic base in (A1) or (A2) units of the oligomer chain, together with the predominant *N*-conformation of pentofuranose residues, is obviously required to form a productive complex between the enzyme, mediator, and substrate. The s*yn*-orientation of the base in (A3) moiety of the oligomer is also positive for RNase L activation. The trimer stereochemical features have been established to be pivotal in shaping RNase L binding and activation rather than the presence of C3′-OH group in 2-5A (A2) moiety.

3.2 Influence of structural (stereochemical) features of 3′-fluoroanalogs 2-5A on human NK cell lytic activity

The 2-5A system is not probably responsible for all interferon (INF) biological effects, however, varied evidence suggest that 2-5A, as well as 5′-dephosphorylated analogs are involved in various biochemical processes in animal cells including INF antiviral effect [35]. The INF ability to regulate lytic activity of parent killer cells (NK cells) [36, 37] is considered one of the key factors of INF antitumor effect [38]. It is noteworthy that NK cells are responsible for lysis of virtually any tumor and virally infected cells irrespective of antibodies or complement with no prior immunization needed [39, 40].

(2′-5′)Oligoadenylates, similarly to IFN, increase the NK cell lytic activity at an optimum concentration of 50 μM thus mimicking IFN action [41]. The study of effects produced by the parent trimer, A_3, and 3′-deoxyadenosine analog ($3'dA_3$) on NK cells showed that the increased NK cell activity is typical only for oligomers with a (2′-5′) phosphodiester bond. The (3′-5′)oligoadenylic acid trimer, (3′-5′)A_3 produced no effect on NK cell lytic activity even at a concentration of 300 μM. Adenosine and 3′-deoxyadenosine at a concentration of 150 μM also did not change NK cell activity, which rules out the effect of trimers as depot forms of nucleosides. It was assumed that the stereochemistry of (3′-5′)- and (2′-5′) phosphodiester bonds caused differences in the effects on NK cell lytic activity of these oligomers [41].

Based on these data, similar activity of A_3 and $(3'dA)_3$ in respect to NK cells seems unexpected. Indeed, both trimers are widely different in their spatial structure [31, 42]—the parent trimer being a conformationally flexible molecule, and for $(3'dA)_3$, only one spatial structure is predominantly occupied. Apparently, the $(3'dA)_3$ molecule is not more rigid thermodynamically compared to the parent trimer, A_3, and is capable of taking a spatial arrangement similar to that of the parent trimer when interacting with NK cells.

The effect of conformationally different molecules of *xylo* and *ribo* 3′-fluorodeoxyanalogs of (2′-5′)oligoadenylic acid on human parent killer (NK cells) was studied [43, 44]. Treatment of human effector NK cells with fluorodeoxyanalogs 2-5A has been generally shown to result in a significantly augmented cytotoxic activity toward target cells. Moreover, the degree of augmentation in NK cell activity varied significantly and depended on the conformation of the fluorodeoxyanalog (**Table 1**). Stereochemistry of (2′-5′)(A^F)A_2 and (2′-5′)A(A^F)A trimers is determined by the predominant population of the furanose ring N-conformation and *syn*-orientation of the heterocyclic base around the glycosidic bond of A^F fragment. Relevant *ribo*-isomers, (2′-5′)(A_F)A_2 and (2′-5′)A(A_F)A are similar to the parent trimer, (2′-5′)A_3, having *anti*-conformation of all heterobases yet rigid S-conformation of A_F furanose ring. All 2-5A *ribo*-fluorodeoxyanalogs were much more active on NK cells compared to *xylo*-analogs or the parent mediator, which is obviously due to a closer conformational resemblance of *ribo*- fluorodeoxyanalogs with the parent oligomer compared to isomeric *xylo*-analogs.

3.3 Effect of 2-5A fluorodeoxyanalogs on macrophage phagocytic activity

Another type of immune cells, mononuclear phagocytes (MPs) are cells, which are directly engaged in the formation of humoral and cellular immune responses. Their

(2′-5′) Oligoadenylate	LU_{20}*	Increased activity (%)**	LU_{30}*	Increased activity (%)**	LU_{50}*	Increased activity (%)**
Control	11.0	0	7.1	0	2.0	0
A_3	16.2	47	10.0	41	4.5	125
$(A^F)A_2$	8.0	–27	6.7	–5.6	2.0	0
$A(A^F)A$	10.0	–9	7.1	0	3.8	90
$A_2(A^F)$	16.0	46	9.4	32	4.2	108
$(A_F)A_2$	16.6	51	10.0	41	5.0	150
$A(A_F)A$	14.3	30	10.0	41	5.5	175
$A_2(A_F)$	11.5	4.5	8.3	17	4.5	125
$A(A^F)(A_F)$	12.2	11	8.0	13	4.3	115

**One lytic unit was assumed equal to the number of effector cell (natural killers) lysing 20% (LU_{20}), 30% (LU_{30}), and 50% (LU_{50}), respectively, of target cells during the study period. K-562 erythroleukemia cells were used as target cells and labeled with a non-radioactive chelate complex of europium diethylenetriaminepentaacetate (EuDTPA).*
***The percentage of increased lytic activity of N-lymphocytes was calculated by the formula: {[(LE/10^6 treated cells/ LE/10^6 control cells)] - 1} x 100.*

Table 1.
Effect of 3′-fluorodeoxyanalogs on NK-lymphocyte activity.

phagocytic activity entailing absorption and killing of certain types of microorganisms is one of many results of MP functioning.

The study of the effect of 2-5A on MPs showed that 2-5A analogs should have three phosphate residues at the 5′-end and at least three adenosine moieties to increase macrophage phagocytic activity. Moreover, it was found that MPs of various animal species have a 2-5A receptor, which has a high specificity. Substitution of adenosine moiety for inosine did not contribute to binding to the 2-5A receptor and thus did not increase macrophage phagocytic activity [45, 46].

Compound	Concentration		Compound	Concentration	
	1×10^{-10} (M)			1×10^{-6} (M)	
	I_{CL}(%)	relative activity		I_{CL}(%)	relative activity
$pppA_3$	46	1.0	A_3	31	1.0
$ppp(A^F)A_2$	56	1.2	$(A^F)A_2$	27	0.9
$pppA(A^F)A$	37	0.8	$A(A^F)A$	16	0.5
$pppA_2(A^F)$	34.5	0.75	$A_2(A^F)$	21	0.7
$ppp(A_F)A_2$	92	2.0	$(A_F)A_2$	40	1.3
$pppA(A_F)A$	57.5	1.25	$A(A_F)A$	43	1.4
$pppA_2(A_F)$	69	1.5	$A_2(A_F)$	42	1.35
$pppA(A^F)(A_F)$	31	0.67			

Values shown have been obtained from six to nine independent experiments. The phagocytic activity of macrophages was expressed in terms of the chemiluminescence index: $I_{CL}(\%) = \frac{CL_2 - CL_1}{CL_1} \cdot 100$, where CL_1 is the maximum chemiluminescence (mV) of native P388D1 cells and CL2 is the maximum chemiluminescence (mV) of activated P388D1 cells.

Table 2.
Effect of fluorodeoxyanalogs 2-5Aa on the phagocytic activity of mouse macrophages of the P388D1 line.

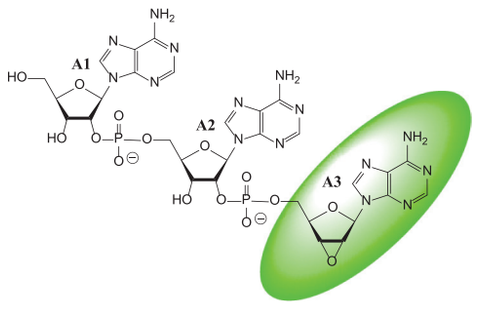
It was demonstrated that all 2-5A *ribo*-fluorodeoxyanalogs were much more superior in activating P388D1 cells than *xylo*-analogs, and the parent oligomer [47, 48]. Probably, *anti*-conformation of the heterocyclic base with the dominant S-conformation of carbohydrate moieties is more preferable for 2-5A receptor of P388D1 cells compared to the parent trimer where the furanose ring is located in the dynamic S↔N equilibrium with prevalent *anti*-orientation of adenine bases [31]. Conformational rigidity of the carbohydrate ring of the xylonucleoside, A^F, which was found to be largely occupied in *N*-conformation along with *syn*-orientation of the base resulted in reduced activation of P388D1 cells induced by (2′-5′) oligoadenylates. However, significant differences exist between *xylo*-and *ribo*-analogs in their ability to increase phagocytic activity (**Table 2**).

4. (2′-5′)oligoadenylates containing *epoxy*-groups rather than 2′,3′-cis-diol group

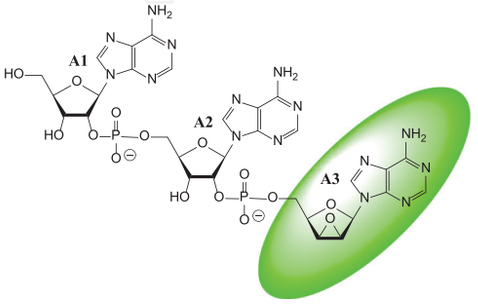
The use of 2-5A analogs in kidney transplant surgeries in monkeys was demonstrated in [49]. Based on the results, 2-5A analogs were selected from a variety of synthesized compounds in which 2′(3′)-terminal adenosine moiety of the molecule was substituted with 9-(2,3-anhydro-β-D-ribofuranosyl)adenine [(2′,5′)A_2A_{RA}] or 9-(2,3-anhydro-β-D-lyxofuranosyl)adenine [(2′,5′)A_2 A^{LA}] (**Figure 4**).

These compounds (*i*) were more resistant to snake venom phosphodiesterase compared to the parent trimer, (2′,5′)A_3, ($t_{1/2}$ hydrolysis 16 h, 10.5 h, and 27 min, respectively), *(ii)* exhibited no toxicity in mice at a concentration of 1 mg per kg of body weight and *(iii)* showed peculiar characteristics of lymphocytes in blastogenic response in the presence of mitogens during the *in vitro* experiments (**Tables 3** and **4**). The effects produced by these isomeric analogs were much more pronounced and they differed from 2′-5′A_3 in their response to concanavalin A (Con A)- and lipopolysaccharide (LPS)-induced lymphocyte blastogenic response (**Table 3**). Con A primarily stimulates T-lymphocyte division whereas LPS acts on β-lymphocytes.

Biochemical test results convincingly demonstrated the potential of two analogs (2′,5′)A_3, (2′,5′)$A_2A_{RA,}$ and (2′,5′)A_2A^{LA} for the treatment of various conditions including those associated with T-cell immunodeficiency diseases, autoimmune diseases, viral infections, lymphocytic malignant transformations, graft failure prevention following the bone marrow transplantation and perhaps, complications associated with the recipient's transplant rejection.



(2',5')A_2A_{RA}



(2',5') A_2A^{LA}

Figure 4.
2-5A dephosphorylated analogs containing epoxy-groups rather than 2′,3′-cis-diol group in A3 moiety in ribo-[(2′,5′)A_2A_{RA}] and lyxo-(2′,5′)A_2A^{LA}] configurations.

Mitogen	DNA synthesis by incorporation of [^{3}H]-thymidine (pulse/min)			
	Control	Connection (5×10^{-6} M)		
		(2′,5′)A_3	(2′,5′)A_2A_{RA}	(2′,5′) A_2A^{LA}
Con A (5 μg/mL)	22.296	18.934	16.399	15.749
LPS (0.1 μg/mL)	27.133	1.588	21.464	10.828

Table 3.
Comparative analysis of in vitro mammalian lymphocyte blastogenic response.

Control	DNA synthesis by incorporation of [^{3}H]-thymidine (pulse/min)				
	Concentration (2′,5′)A_2A_{RA} (M)*				
	5×10^{-6}	5×10^{-7}	5×10^{-8}	5×10^{-9}	5×10^{-10}
Con A (5 μg/mL) 5.428 *[5.108]*	1.733 *[7732]*	3.865 *[2237]*	5.383 *[1355]*	1.599 *[6915]*	777 *[1425]*
LPS (0.1 μg/mL) 10.766 *[5.513]*	7.665 *[131]*	6.018 *[292]*	4.767 *[655]*	7.348 *[3342]*	672 *[1213]*

**Data for (2′,5′)A_3 at the same concentration are shown in square brackets.*

Table 4.
Concentration dependence of in vitro lymphocyte blastogenic response as affected by (2′,5′)A_2A_{RA} vs. (2′,5′)A_3.

The potential use of (2′,5′)A_2A_{RA} and (2′,5′)A_2A^{LA} analogs for kidney transplantation in rabbits and monkeys has been thoroughly studied [49]. Daily intravenous injection of (2′,5′) A_2A_{RA} to rabbits at a dose of 5 μg/kg of body weight ensures the normal functioning of the transplanted kidney in 4 out of 10 animals within 3 months. The lymphocyte blastogenic response in positive rabbits was suppressed by about 10 times by concanavalin (Con A) stimulation within 2 weeks after surgery.

Immunotropic activity of (2′,5′)A_2A_{RA} and (2′,5′)A_2A^{LA} analogs vs. (2′,5′)A_3 was assessed in a group of monkeys aged 4. The parent trimer (2′,5′)A_3 showed no immunosuppressive activity at a concentration of 0.5 mg/kg (data not shown). Moreover, a single intravenous injection resulted in a ~ 50% increase in T-helpers and T-killers responsible for the transplant rejection.

The results of the (2′,5′)A_2A_{RA} study are summarized in **Table 5**. It should be emphasized that the analog inhibits interleukin-2 (IL-2) and T-lymphocyte subpopulation and concurrently stimulates αIFN and γIFN in blood lymphocytes for 2–3 weeks with a single injection at a concentration of 50 μg/kg (**Table 5**) and 25 μg/kg (data not shown).

After cross-allotransplantation of the kidney in two groups of monkeys, they were followed up for 3 months. Of note, normal functioning of the transplanted kidney occurred within 10 hours after the operation.

The data shown in **Table 5** suggest selective suppression of T-lymphocyte subpopulation immediately after surgery, subsequent recovery to preoperative levels followed by mild reduction of T-helper and T-killer populations, and slight increase in T-suppressor count. This trend persisted throughout the postoperative follow-up period.

Experiments in monkeys have shown that intravenous (2′-5′)A_2A_{RA} given every 48 hours at a concentration of 50 μg/kg provides immunosuppression, protects the

Type of assay	Days after the introduction of the trimmer					
	0	**1**	**2**	**8**	**12**	**21**
IgG (g/L)	9.6	7.8	15.0	14.8		13.2
IgA (g/L)	2.8	2.7	0.8	0.9		0.4
IgM (g/L)	0.6	0.5	1.1	0.9		0.8
T-helpers (%)	30	29	15	5.0	7.0	30.6
T-suppressors (%)	43	31	19	7.4	13	44
T-killers (%)	12	9	7	4.4	9	19.3
αIFN*) (plasma)	16	32	4	—	4	4
γIFN*) (lymphocytes)	4	8	32	—	32	8
αIFN*) (lymphocytes)	32	32	64	—	64	32
IL- 2	8	4	4	—	4	4

Units of IFN per 10.000 lymphocytes.

Table 5.
Effect of a single intravenous injection of trimer (2′,5′)A_2A_{RA} at a dose of 50 μg/kg on the immune system of macaque Rh monkeys.

Type of assay	2 days pre-oper. 50 μg/kg	Days postoperation				
		1	**5**	**8**	**13**	**18**
IgG (g/L)	13	—	12.2	12.8	—	—
IgA (g/L)	0.4	—	2.1	2.5	—	—
IgM (g/L)	0.8	—	0.8	0.9	—	—
T-helpers (%)	30	8	39.9	32	34.7	14.5
T-suppressors (%)	44	40.6	44	47	47.7	57.5
T-killers (%)	19	5	19	22	22.6	16

Table 6.
Effect of intravenous administrations of (2′-5′)A_2A_{RA} (50 μg/kg) on the immune system of macaque Rh. Monkeys after kidney transplantation. Intravenous administration (50 μg/kg) on day 2, 6, and 12 after surgery and every sixth day thereafter.

graft from rejection, and resumes normal functioning of the kidney transplant. T-helper and T-killer counts during the first two most critical postoperative weeks were reduced by 2–3 times remaining at 30% of a normal value (**Table 6**).

5. Conclusions

As a result of studies to clarify the relationship between the structure and spatial organization (stereochemistry), on the one hand, and the biological properties of analogs 2-5A, on the other one, it was convincingly shown that by changing the structure and/or stereochemistry of their molecules, it is possible to achieve either strengthening of known or giving new properties. The different functional groups of each individual 2-5A nucleotide fragment make a highly specific contribution to the binding and activation of RNase L, as well as to hydrolytic stability.

The use of conformationally rigid 3′-fluorodeoxyanalogs 2-5A and core trimer 2-5A on the example of human NK lymphocytes and mouse macrophages of the $P388D_1$ line allows us to evaluate the influence of structural and stereochemical factors on the cells of the immune system. *Ribo*-fluorodeoxyanalogs 2-5A had a more significant activating effect on human NK lymphocytes and phagocytic activity than *xylo*-analogs or a natural mediator, which is probably due to the closer conformational similarity of *ribo*-fluorodeoxyanalogs with a natural oligomer than with isomeric *xylo*-analogs.

Analogs 2-5A, $(2',5')A_2A_{RA}$, and $(2',5')A_2A^{LA}$ are undoubtedly of considerable interest for transplantology as drugs that prevent kidney rejection, ensure the normal functioning of the transplanted kidney and at the same time do not increase the level of T-helper and T-killer cells in experimental animals in postoperative period.

Undoubtedly, the search for approaches to the directed regulation of the natural protective function of the body with the help of analogs of the core trimer 2-5A can lead to the detection of compounds with high therapeutic potential.

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